FORM PTO-1390 (Modified) (REV 11-2000) U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER	U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR)
101195-67	09/979513

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/DE00/01444	10 May 2000 (10.05..00)	14 May 1999 (14.05.99)

TITLE OF INVENTION

Method for Detecting the Effectof Different Chemotherapeutic Agents and/or Radiation Therapy in Malignant Diseasaes and Method for Selecting More Effective Therapeutic Agents for the Therapy Thereof

APPLICANT(S) FOR DO/EO/US

Peter Daniel; Timo Hillebrand; Bernd Dorken; and Peter Bendzko

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This is an express request to begin national examination procedures (35 U.S.C. 371(f)). The submission must include itens (5), (6), (9) and (24) indicated below.
4. ☒ The US has been elected by the expiration of 19 months from the priority date (Article 31).
5. ☒ A copy of the International Application as filed (35 U.S.C. 371 (c) (2))
   a. ☐ is attached hereto (required only if not communicated by the International Bureau).
   b. ☒ has been communicated by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☒ An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).
   a. ☒ is attached hereto.
   b. ☐ has been previously submitted under 35 U.S.C. 154(d)(4).
7. ☐ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371 (c)(3))
   a. ☐ are attached hereto (required only if not communicated by the International Bureau).
   b. ☐ have been communicated by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☐ have not been made and will not be made.
8. ☐ An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☐ An oath or declaration of the inventor(s) (35 U.S.C. 371 (c)(4)).
10. ☐ An English language translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371 (c)(5)).
11. ☐ A copy of the International Preliminary Examination Report (PCT/IPEA/409).
12. ☐ A copy of the International Search Report (PCT/ISA/210).

Items 13 to 20 below concern document(s) or information included:

13. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
14. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
15. ☒ A **FIRST** preliminary amendment.
16. ☐ A **SECOND** or **SUBSEQUENT** preliminary amendment.
17. ☐ A substitute specification.
18. ☐ A change of power of attorney and/or address letter.
19. ☐ A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.2 and 35 U.S.C. 1.821 - 1.825.
20. ☐ A second copy of the published international application under 35 U.S.C. 154(d)(4).
21. ☐ A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4).
22. ☒ Certificate of Mailing by Express Mail
23. ☒ Other items or information:

    Application Data Sheet

U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR)	INTERNATIONAL APPLICATION NO.	ATTORNEY'S DOCKET NUMBER
09/979513	PCT/DE00/01444	101195-67

24. The following fees are submitted:.

BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)) :

		CALCULATIONS PTO USE ONLY
☐	Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO **$1040.00**	
☒	International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO **$890.00**	
☐	International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO **$740.00**	
☐	International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4) **$710.00**	
☐	International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4) **$100.00**	
	ENTER APPROPRIATE BASIC FEE AMOUNT =	**$890.00**
	Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☒ 30 months from the earliest claimed priority date (37 CFR 1.492 (e)).	**$130.00**

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE	
Total claims	10 - 20 =	0	x $18.00	**$0.00**
Independent claims	2 - 3 =	0	x $84.00	**$0.00**
Multiple Dependent Claims **(check if applicable)**.			☐	**$0.00**

TOTAL OF ABOVE CALCULATIONS =	**$1,020.00**
☒ Applicant claims small entity status. See 37 CFR 1.27). The fees indicated above are reduced by 1/2.	**$510.00**
SUBTOTAL =	**$510.00**
Processing fee of **$130.00** for furnishing the English translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492 (f)). +	**$0.00**
TOTAL NATIONAL FEE =	**$510.00**
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31) **(check if applicable).** ☐	**$0.00**
TOTAL FEES ENCLOSED =	**$510.00**
Amount to be: refunded	$
charged	$

a. ☐ A check in the amount of _____ to cover the above fees is enclosed.

b. ☒ Please charge my Deposit Account No. **14-1263** in the amount of **$510.00** to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. **14-1263** A duplicate copy of this sheet is enclosed.

d. ☐ Fees are to be charged to a credit card. **WARNING:** Information on this form may become public. **Credit card information should not be included on this form.** Provide credit card information and authorization on PTO-2038.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

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27387
PATENT TRADEMARK OFFICE

SIGNATURE

Bruce S. Londa
NAME

33,531
REGISTRATION NUMBER

November 14, 2001
DATE

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
Atty's Docket No. 101195-67

APPLICANT : Peter Daniel et al.

FILED : Concurrently Herewith

FOR : Method for Detecting the Effect of Different Chemotherapeutic Agents and/or Radiation Therapy in Malignant Diseases and Method for Selecting More Effective Therapeutic Agents for the Therapy Thereof

PRELIMINARY AMENDMENT

Hon. Assistant Commissioner of Patents
Washington, D.C. 20231

Sir:

Prior to examination, please amend the application as follows:

IN THE SPECIFICATION

Page 1, after line 4, please insert --Background of the Invention--;

Page 2, after line 16, please insert --Summary of the Invention--;

Page 4,after line 3, please insert --Brief Description of the Drawings

Fig. 1 - grafts showing the association of the level of Bax and the ex-vivo response of CLL patients to a number of cytostatic agents. The figure shows the relative level of Bax protein expression (densitometric values from Western blot analyses) and the LC90 dose for each of the cytostatic agents;

Fig. 2 - grafts showing the association between Bax expression and the LC90 dose of doxorubicin in 37 CLL patients. This relationship with regard to the cytotoxic effect of the cytostatic agent could only be observed for Bax, but not for Bcl-2 or the ratio of Bcl-2 to Bax;

Fig. 3 - grafts showing the reduced sensitivity to cytostatic agents (height of the bars corresponds to the dose of the cytostatic in µg/ml) for p53-mutant CLL patients compared to the p53 wild type. The p53 mutations were determined for the exons 5 to 8 using SSCP-PCR.

Detailed Description of the Preferred Embodiments--;

Page 11, please delete this page in its entirety.

IN THE CLAIMS

Please amend the claims in accordance with the attached marked-up pages. A clean copy of the amended claims is also enclosed.

REMARKS

The above amendments were made to place the application into proper United States Patent Format.

Respectfully Submitted,

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1. Method for detecting the effect of different chemotherapeutic agents and/or radiation therapy in malignant diseases, wherein the expression profiles of apoptosis-regulating and/or cell growth regulating genes and/or individual differences (mutations) in the gene sequences is determined and the changes associated with chemotherapeutic agents and/or radiation therapy are identified, represented and diagnostically evaluated.

2. Method in accordance with claim 1, wherein the expression profiles of the genes of the Bcl-2 family, preferably Bax, p53, p16, caspases, Rb , cyclins, inhibitors of cyclin-dependent kinases (CDKIs), ATM and inhibitors of apoptosis proteins (IAPs) and/or mutations in the genes are determined using protein or DNA/RNA analyses and evaluated singly or in various combinations.

3. (amended) Method in accordance with claim 1~~ or 2~~, wherein individual differences in the sequence of apoptosis and/or cell growth-regulating genes and or the expression of their gene products, which occur in malignant diseases, are related to an individually different responsiveness to drugs and are evaluated, particularly with regard to their relevance to the response to therapy.

4. Method for selecting more efficacious therapeutic agents for the treatment of malignant diseases, wherein the status of cell cycle genes and/or of apoptosis-associated target genes or of their gene products in body fluids, cells or organs are determined ex vivo and the more efficacious agents for this status are selected.

5. Method in accordance with claim 4, wherein agents for the treatment of leukemic diseases and other hematological

malignomas and solid tumors like, for example, tumors of the gastrointestinal tract, pancreas, prostate, gynecological tumors, sarcomas, brain tumors, skin and lung tumors as well as tumors of endocrine organs are evaluated.

6. (amended) Method in accordance with claim 4 ~~or 5~~, wherein therapeutic agents are known cytostatic agents, preferably steroid hormones, alkylating agents, anthracyclines, antimetabolites, taxanes, topoisomerase inhibitors, Vinca alkaloids, cisplatin and other platinum analogues and many more.

7. (amended) Method in accordance with claim 1 ~~to 6~~, wherein for the treatment of solid tumors or leukemic or other hematological malignant diseases, preferably of chronic lymphocytic leukemia, the Bax expression or mutations are evaluated and, with a low Bax expression, a treatment with alkylating agents, anthracyclines and Vinca alkaloids is avoided and another form of therapy is chosen.

8. (amended) Method in accordance with claim 1 ~~to 6~~, wherein for the treatment of leukemic diseases, mainly of chronic lymphocytic leukemia, the Bax expression or mutations are evaluated and, with low Bax expression, a treatment with steroid hormones or fludarabine phosphate (2-CDA) is carried out.

9. (amended) Method in accordance with claim 1 ~~to 6~~, wherein for the treatment of leukemic diseases, mainly of chronic lymphocytic leukemia, the p53 expression or mutations are evaluated and, with the presence of mutations within the coding sequence regions of the p53 genes, a treatment with DNA-damaging substances, particularly with alkylating agents,

anthracyclines and fludarabine, is avoided and another form of therapy is selected.

10. (amended) Method in accordance with claim 1~~ to 9~~, wherein by combination of the determination of the status of different apoptosis and/or cell growth-associated genes, mainly of p53 and Bax or their gene products and/or mutations and/or their homologues, individual schemes of treatment are drawn up.

~~11.Use of the status determination via protein or DNA/RNA analyses of apoptosis and/or cell growth-associated genes or their gene products for determination of resistances to radiation therapy and to therapeutic agents as well as for the purposeful selection of therapeutic agents for cytotoxic therapies.~~

~~12.Use in accordance with claim 11, wherein the status, namely the expression profile and/or mutations of genes of the Bcl-2 family, mainly Bax, p53, p16, caspases, Rb, cyclins, inhibitors of the cyclin-dependent kinases (CDKIs), ATM and inhibitors of the apoptosis proteins (IAPs), of individual genes or in different combinations is determined.~~

~~13.Use in accordance with claim 12, wherein the status of Bax expression or mutations of the Bax gene or Bax promoters and Bax regulators is used.~~

~~14.Use in accordance with claim 12, wherein the status of the p53 expression or mutations of the p53 gene is used.~~

~~15.Use in accordance with claim 11 to 14, wherein the status of Bax and p53 risk-adapted tumor therapies with malignant hematological diseases, like CLL, and other tumoral diseases is used.~~

~~16.Use in accordance with claim 11 to 15 of a combination of p53 and Bax and possibly other cell cycle and apoptosis regulators either alone or in their combination in the sense of a molecular pathway diagnosis with malignant tumors or precancers.~~

Claims - Clean Copy

1. Method for detecting the effect of different chemotherapeutic agents and/or radiation therapy in malignant diseases, wherein the expression profiles of apoptosis-regulating and/or cell growth regulating genes and/or individual differences (mutations) in the gene sequences is determined and the changes associated with chemotherapeutic agents and/or radiation therapy are identified, represented and diagnostically evaluated.

2. Method in accordance with claim 1, wherein the expression profiles of the genes of the Bcl-2 family, preferably Bax, p53, p16, caspases, Rb , cyclins, inhibitors of cyclin-dependent kinases (CDKIs), ATM and inhibitors of apoptosis proteins (IAPs) and/or mutations in the genes are determined using protein or DNA/RNA analyses and evaluated singly or in various combinations.

3. (amended) Method in accordance with claim 1, wherein individual differences in the sequence of apoptosis and/or cell growth-regulating genes and or the expression of their gene products, which occur in malignant diseases, are related to an individually different responsiveness to drugs and are evaluated, particularly with regard to their relevance to the response to therapy.

4. Method for selecting more efficacious therapeutic agents for the treatment of malignant diseases, wherein the status of cell cycle genes and/or of apoptosis-associated target genes or of their gene products in body fluids, cells or organs are determined ex vivo and the more efficacious agents for this status are selected.

5. Method in accordance with claim 4, wherein agents for the treatment of leukemic diseases and other hematological

malignomas and solid tumors like, for example, tumors of the gastrointestinal tract, pancreas, prostate, gynecological tumors, sarcomas, brain tumors, skin and lung tumors as well as tumors of endocrine organs are evaluated.

6. (amended) Method in accordance with claim 4, wherein therapeutic agents are known cytostatic agents, preferably steroid hormones, alkylating agents, anthracyclines, antimetabolites, taxanes, topoisomerase inhibitors, Vinca alkaloids, cisplatin and other platinum analogues and many more.

7. (amended) Method in accordance with claim 1, wherein for the treatment of solid tumors or leukemic or other hematological malignant diseases, preferably of chronic lymphocytic leukemia, the Bax expression or mutations are evaluated and, with a low Bax expression, a treatment with alkylating agents, anthracyclines and Vinca alkaloids is avoided and another form of therapy is chosen.

8. (amended) Method in accordance with claim 1, wherein for the treatment of leukemic diseases, mainly of chronic lymphocytic leukemia, the Bax expression or mutations are evaluated and, with low Bax expression, a treatment with steroid hormones or fludarabine phosphate (2-CDA) is carried out.

9. (amended) Method in accordance with claim 1, wherein for the treatment of leukemic diseases, mainly of chronic lymphocytic leukemia, the p53 expression or mutations are evaluated and, with the presence of mutations within the coding sequence regions of the p53 genes, a treatment with DNA-damaging substances, particularly with alkylating agents, anthracyclines and fludarabine, is avoided and another form of therapy is selected.

Claims - Clean Copy

10. (amended) Method in accordance with claim 1, wherein by combination of the determination of the status of different apoptosis and/or cell growth-associated genes, mainly of p53 and Bax or their gene products and/or mutations and/or their homologues, individual schemes of treatment are drawn up.

Method for detecting the effect of different chemotherapeutic agents and/or radiation therapy in malignant diseases and method for selecting more effective therapeutic agents for the therapy thereof

The invention relates to a method for detecting the effect of different chemotherapeutic agents and/or radiation therapy in malignant diseases, wherein the expression profile of tumor and/or cell growth and/or apoptosis-associated genes and/or individual differences (mutations) in gene sequences are determined. Changes associated with chemotherapeutic agents and/or radiation therapy are identified, represented and diagnostically evaluated. The invention further relates to a method for selecting more effective therapeutic agents for the therapy of malignant diseases. The status of cell cycle genes and/or apoptosis-associated target genes or gene products thereof in body fluids, cells and/or organs is determined and diagnostically evaluated to determine their effect on corresponding therapeutic agents. In a preferred embodiment, Bax and p53 expressions or mutations are investigated and the findings therefrom are used for deciding individual-specific therapy in leukemia and other malignant diseases.

The process of a malignant change of a cell begins very early, often with only a single change in the genetic material. It goes through different stages until it produces the degenerated cell and is not yet complete even at this stage.

Progress in modern molecular biology, accompanied by a better understanding of the development of malignant changes on the molecular level, has yielded a multitude of new types of information on the factors involved in the process of carcinogenesis and tumor progression. However, these findings also clearly show how varied, different and complex changes in the molecular network are in order to ultimately be manifested as

a malignant phenotype or therapy-resistant tumor. The involvement of various factors in the process of tumor development is, on the one hand, an indication of the complexity of such a process, but they can also be utilized for diagnostic purposes as well as for a prognostic risk assessment.

In spite of the state of knowledge which has been achieved until now, the staging and the treatment of tumor patients based on it continues to be carried out according to histopathological or clinical criteria. This conventional classification is therefore still decisive for making all therapeutic decisions (Hämatologie Onkologie, ed. P.C. Ostendorfund S. Seeber, Urban and Schwarzenberg 1997; Kompendium Internistische Onkologie, ed. H.J. Schmoll, K. Höffken, K. Possinger, Springer 1997).

Until now, an individual-specific treatment based on a maximally extensive molecular characterization of the tumor could not be carried out.

Therefore, the purpose of the invention is to use findings on the molecular level for an individual-specific tumor therapy and for finding an effective selection of therapeutic agents in other to provide affected patients with an efficacious treatment.

Tumorigenesis, tumor progression and resistance to therapy are determined by cell cycle and apoptosis-regulating factors. The present invention was based on the unexpected finding that by determining their expression profile, one can use these tumor or cell growth-associated genes as prognostic markers and can correlate them with the response of the patient to different chemotherapeutic agents, radiation therapy and clinical parameters. Based on the obtained marker profile, an effective and promising form of therapy can then be derived for the patients.

The invention therefore relates to a method for detecting the effect of different chemotherapeutic agents and/or radiation therapy in malignant diseases. In accordance with the invention, the expression profiles of tumor and/or cell growth-associated genes and/or individual differences (mutations) in the gene sequences are determined and interactions (associations) with chemotherapeutic agents and/or radiation therapy are identified, represented and diagnostically evaluated.

It has been established that the disturbance of apoptosis together with the deregulation of the cell cycle is a decisive mechanism with regard to the development, growth and progression of malignant tumors. The restoration of the capability of cell cycle arrest and of apoptosis-promoting genes is already an important goal of experimental gene therapeutic strategies. It was found than an inhibition of apoptosis signal cascades can lead to resistance against cytostatic and radiation therapy, which is of enormous clinical importance above all with solid tumors. These resistance mechanisms can originate from disturbances of apoptosis and the cell cycle in that pathologically relevant changes are contained in specific genes and their gene products. Important tumor genes which are responsible for resistance mechanisms are, for example, the genes of the Bcl-2 family, mainly Bax, p53, p16, caspases, Rb, cyclins, inhibitors of cyclin-dependent kinases (CDKIs), ATM and inhibitors of apoptosis proteins (IAPs).

In accordance with the invention, it is preferable to determine the expression profiles and/or mutations of the above-mentioned genes by means of protein or DNA/RNA analyses. During this process, the expression profiles and/or mutations of the respective individual genes are evaluated. But the profiles and/or mutations of different genes can also be combined, whereby the drawing up of individual schemes of treatment is improved and an individual prognosis and risk assessment is possible. Preferably, expression profiles of Bax, p53, p16, caspase and/or

Rb genes or their mutations will be used and diagnostically evaluated. It is especially preferable to identify the status of p53 genes and Bax genes or of their gene products.

This invention particularly concerns a method for selecting more effective therapeutic agents for the treatment of malignant diseases. It is characterized by the determination of the status of cell cycle genes and/or apoptosis-associated target genes or their gene products ex vivo in body fluids, cells and organs, which is diagnostically evaluated in connection with the effect of corresponding therapeutic agents.

Therapeutic agents in accordance with the invention are known agents for the treatment of leukemic or lymphoma diseases and of other malignant diseases like, for example, tumors of the gastrointestinal tract, pancreas, prostate, gynecological tumors (e.g. ovary, cervix, breast), sarcomas, brain tumors, skin and lung tumors as well as tumors of endocrine organs like, for example, the thyroid etc.

These include the well-known cytostatic agents, mainly steroid hormones (e.g. prednisone, prednisolone, methylprednisolone, and other glucocorticoids), antimetabolites (cladribine (2-CDA), fludarabine, mercaptopurine, arabinoside C, 5-substituted dideoxynucleosides, like 5-fluoruracil, azidothymidine), alkylating agents (e.g. mafosfamide, chlorambucil, melphalan, cyclophosphamide), taxanes (like paclitaxel, docetaxel), anthracyclines (e.g. idarubicin, doxorubicin, epirubicin, mitoxanthrones), topoisomerase inhibitors (like etoposide), Vinca alkaloids, (vincristine, vinblastine, vinorelbine), cisplatin and other platinum analogues and many more as well as radiation therapy.

The invention will be explained in more detail below using the example of leukemic diseases. For example, it was established that particularly for the therapy of chronic lymphocytic leukemia

(CLL), the analysis of the Bax expression or mutations showed that with loss of Bax expression a treatment with alkylating agents, anthracyclines and Vinca alkaloids is far less effective. Therefore, such a therapy should be avoided and other forms of therapy should be preferred. The data on which the invention are based clearly show that in patients with CLL and a low Bax expression, the response to therapy with cytostatic agents in vitro is very poor compared with the alkylating agents, mafosfamide, chlorambucil, and melphalan, the anthracyclines, doxorubicin and epirubicin, and the Vinca alkaloid, vincristine. This in vitro sensitivity data correlate with the response to therapy in vivo.

On the other hand, it could be shown that for the treatment of leukemic diseases, chiefly CLL, with low Bax expression, an in vitro therapy with steroid hormones or fludarabine is promising. There is in fact no influence on the responsiveness to steroid hormones (e.g. prednisolone and methylprednisolone) or to fludarabine.

This in vitro data on the chemosensitivity of CLL cells show a good correlation with the clinical response of the patients to the substances in vivo.
Furthermore, they also provide evidence that the diagnostic clarification of the Bax status is essential for making a therapy-related decision. Since the loss of Bax expression can take place at various stages of the realization of genetic information, various detection methods must be included in such a diagnostic procedure. This applies, for example, to the detection of mutative changes on the DNA level (e.g. point mutations, frame-shift mutations) and the detection of hypermethylation patterns within the Bax promoter region as a possible cause for a transcriptional silencing and tests of the expression or expression level of the Bax protein (immunohistochemistry or Western blot or flow cytometry or other quantitative detection

methods) as well as the analysis of transcriptional and translational regulators and the protein breakdown.

The evaluation of the p53 expression or of mutations of the p53 gene has shown that for the treatment of leukemic diseases, particularly of CLL, a therapy with DNA-damaging substances, especially with alkylating agents, anthracyclines and fludarabine, should be avoided if mutations are present within the coding sequence regions of the p53 gene. With regard to a positive correlation of mutations of the tumor suppressor protein p53 and a differential response to cytostatic agents, it could be shown that for patients with mutations within the coded sequence regions of the p53 gene, the response is significantly poorer to fludarabine and DNA-damaging substances like, for example, the alkylating agents. Therefore, for the candidate gene p53 as well, an extensive diagnostic analysis must be carried out on the DNA and protein level. In principle, that also applies for the p53-homologous candidate genes p73 and p41.

Ultimately, via the combined diagnosis of the two candidate genes p53 and Bax, an individualized form of therapy can be applied by drawing up individual schemes of treatment based on the combination of the genetic status of the p53 and Bax genes or of their gene products and/or mutations.

Furthermore, the invention relates to the use of status determination of cell cycle genes and/or apoptosis-associated target genes or their gene products or mutations using protein or DNA/RNA analysis for the determination of the resistance to therapy and for the specific selection of therapeutic agents for cytotoxic therapies. Preferably, the analysis is carried out based on Bax expression or mutations or based on p53 expression or mutations.

Special priority is given to the use of status determination of Bax and p53 genes for a risk-adapted tumor therapy with leukemic

diseases like CLL and other tumors. In another concrete form of the invention, the use occurs in combination of p53 and Bax with other cell cycle and apoptosis regulators, which are also employed either alone or in combination as a molecular pathway (signal pathway) diagnosis with malignant tumors or precancers.

Although it is known that due, for example, to an existing clear functionality of the tumor suppressor protein p53 within the cell cycle and apoptosis regulation, a loss of p53 function is often found in tumors (e.g. via mutation, deletion or promoter changes). It was surprising, however, that a functionally damaged p53 protein nevertheless facilitates a selective response to certain cytostatic agents in patients with chronic lymphocytic leukemia (CLL). That could be proven for the first time. In addition, this also concerns a multitude of other apoptosis-associated target genes and especially their functional combination.

The present investigations were carried out as an example for the tumor suppressor protein p53 and the proapoptotic gene Bax in patients with chronic lymphocytic leukemia (CLL). Additional data are also available on, for example, these and other apoptosis and cell cycle regulators with tumors of the gastrointestinal tract like, for example, gastric carcinoma, esophageal carcinoma and sarcomas.

With the present invention, conclusive evidence could be furnished based on the two apoptosis-influencing proteins p53 and Bax that via a diagnostic characterization of relevant tumor genes on the molecular level (DNA) and on the expression level (protein) that the possibility exists of selectively choosing cytostatic agents in order to achieve an improved treatment. The findings on molecular pathogenesis and resistance to therapy of tumors can, in accordance with the invention, be used as a basis for an individual-specific tumor therapy and, in this way, result

in a more specific and ultimately also maximally successful treatment for the affected patients.

Thus, one can spare patients who have, according to molecular standards, a good prognosis of responding to the cytotoxic therapies and showing improved survival after therapy elaborate and cost-intensive treatments. At the same time, more aggressive treatments can be carried out in patients with a poorer risk profile in order to improve the therapeutic effect. Furthermore, with detected resistance, a less aggressive treatment can be carried out to avoid unnecessary costs and discomfort for patients.

By means of the invention, it is possible to use changed cellular tumor markers as a decision-making criterion for the selection of various standard chemotherapeutic agents; this is, to also take advantage of positive correlations between changed tumor markers and the efficacy or inefficacy of chemotherapeutic agents. This offers the possibility, after a characterization of selected cellular tumor markers, to selectively chose the form of the chemotherapeutic agent, radiation therapy, or their combination which is to be employed.

The following table 1 shows the effect of various cytostatic agents for a CLL treatment. It is based on comparative studies of the expression of Bax protein, which is known to be proapoptotic with CLL, and the Bcl-2 protein, which functions to block cell death. It is clear from the table that the response to anthracyclines and alkylating agents (doxorubicin, epirubicin, chlorambucil and mafosfamide) as well as vincristine is highly correlated with the level of Bax expression (Western blot) (p-value significant and < 0.05). In contrast, no correlation was found with determination of the Bcl-2 expression. On the other hand, the effect of the steroid hormones (methylprednisolone, prednisolone) and of fludarabine administration showed no correlation to the level of the Bax expression, which indicates

that the agents have an increased cytotoxic effect even on Bax-negative tumor cells.

These conclusions are also confirmed by the correlation of Bax and the ex-vivo response of CLL patients to a number of cytostatic agents which is shown in Figure 1. The figure gives the relative level of Bax protein expression (densitometric values from Western blot analyses) and the LC90 dose for each of the cytostatic agents.

Figure 2 shows the correlation between Bax expression and the LC90 dose of doxorubicin in 37 CLL patients.

Figure 3 shows the reduced sensitivity of CLL cells to cytostatic agents in vitro versus the alkylating agents chlorambucil and melphalan as well as versus fludarabine for p53-mutant CLL patients compared to the p53 wild type. The p53 mutations were determined for the exons 5 to 8 using SSCP-PCR. The height of the bars corresponds to the dose of the cytostatic in µg/ml.

Table 1:
Relationship between the level of the protein expression of Bax, Bcl-2, and the ratio of Bax and Bcl-2 expression (Bcl-2/Bax) and the ex-vivo cytotoxicity of cytostatic agents in the treatment of CLL.

Significance level of the Pearson correlation coefficient (p-value)			
Cytostatic agent	**Bax**	**Bcl-2**	**Bcl-2/Bax**
Fludarabine phosphate	0.816	0.212	0.829
Cladribine (2-CDA)	0.524	0.351	0.658
Chlorambucil	0.034	0.739	0.157
Mafosfamide	0.017	0.250	0.160
Methylprednisolone	0.836	0.415	0.880
Prednisolone	0.807	0.545	0.898
Vincristine	0.011	0.052	0.127
Doxorubicin	0.001	0.920	0.001
Epirubicin	0.002	0.647	0.001

P-values smaller than 0.05 are statistically significant

Figure captions:

Figure 1:
Association of the level of Bax and the ex-vivo response of CLL patients to a number of cytostatic agents. The figure shows the relative level of Bax protein expression (densitometric values from Western blot analyses) and the LC90 dose for each of the cytostatic agents.

Figure 2:
Association between Bax expression and the LC90 dose of doxorubicin in 37 CLL patients. This relationship with regard to the cytotoxic effect of the cytostatic agent could only be observed for Bax, but not for Bcl-2 or the ratio of Bcl-2 to Bax.

Figure 3:
Reduced sensitivity to cytostatic agents (height of the bars corresponds to the dose of the cytostatic in µg/ml) for p53-mutant CLL patients compared to the p53 wild type. The p53 mutations were determined for the exons 5 to 8 using SSCP-PCR.

Patent claims:

1. Method for detecting the effect of different chemotherapeutic agents and/or radiation therapy in malignant diseases, wherein the expression profiles of apoptosis-regulating and/or cell growth regulating genes and/or individual differences (mutations) in the gene sequences is determined and the changes associated with chemotherapeutic agents and/or radiation therapy are identified, represented and diagnostically evaluated.

2. Method in accordance with claim 1, wherein the expression profiles of the genes of the Bcl-2 family, preferably Bax, p53, p16, caspases, Rb , cyclins, inhibitors of cyclin-dependent kinases (CDKIs), ATM and inhibitors of apoptosis proteins (IAPs) and/or mutations in the genes are determined using protein or DNA/RNA analyses and evaluated singly or in various combinations.

3. Method in accordance with claim 1 or 2, wherein individual differences in the sequence of apoptosis and/or cell growth-regulating genes and or the expression of their gene products, which occur in malignant diseases, are related to an individually different responsiveness to drugs and are evaluated, particularly with regard to their relevance to the response to therapy.

4. Method for selecting more efficacious therapeutic agents for the treatment of malignant diseases, wherein the status of cell cycle genes and/or of apoptosis-associated target genes or of their gene products in body fluids, cells or organs are determined ex vivo and the more efficacious agents for this status are selected.

5. Method in accordance with claim 4, wherein agents for the treatment of leukemic diseases and other hematological

malignomas and solid tumors like, for example, tumors of the gastrointestinal tract, pancreas, prostate, gynecological tumors, sarcomas, brain tumors, skin and lung tumors as well as tumors of endocrine organs are evaluated.

6. Method in accordance with claim 4 or 5, wherein therapeutic agents are known cytostatic agents, preferably steroid hormones, alkylating agents, anthracyclines, antimetabolites, taxanes, topoisomerase inhibitors, Vinca alkaloids, cisplatin and other platinum analogues and many more.

7. Method in accordance with claim 1 to 6, wherein for the treatment of solid tumors or leukemic or other hematological malignant diseases, preferably of chronic lymphocytic leukemia, the Bax expression or mutations are evaluated and, with a low Bax expression, a treatment with alkylating agents, anthracyclines and Vinca alkaloids is avoided and another form of therapy is chosen.

8. Method in accordance with claim 1 to 6, wherein for the treatment of leukemic diseases, mainly of chronic lymphocytic leukemia, the Bax expression or mutations are evaluated and, with low Bax expression, a treatment with steroid hormones or fludarabine phosphate (2-CDA) is carried out.

9. Method in accordance with claim 1 to 6, wherein for the treatment of leukemic diseases, mainly of chronic lymphocytic leukemia, the p53 expression or mutations are evaluated and, with the presence of mutations within the coding sequence regions of the p53 genes, a treatment with DNA-damaging substances, particularly with alkylating agents, anthracyclines and fludarabine, is avoided and another form of therapy is selected.

10. Method in accordance with claim 1 to 9, wherein by combination of the determination of the status of different

apoptosis and/or cell growth-associated genes, mainly of p53 and Bax or their gene products and/or mutations and/or their homologues, individual schemes of treatment are drawn up.

11. Use of the status determination via protein or DNA/RNA analyses of apoptosis and/or cell growth-associated genes or their gene products for determination of resistances to radiation therapy and to therapeutic agents as well as for the purposeful selection of therapeutic agents for cytotoxic therapies.

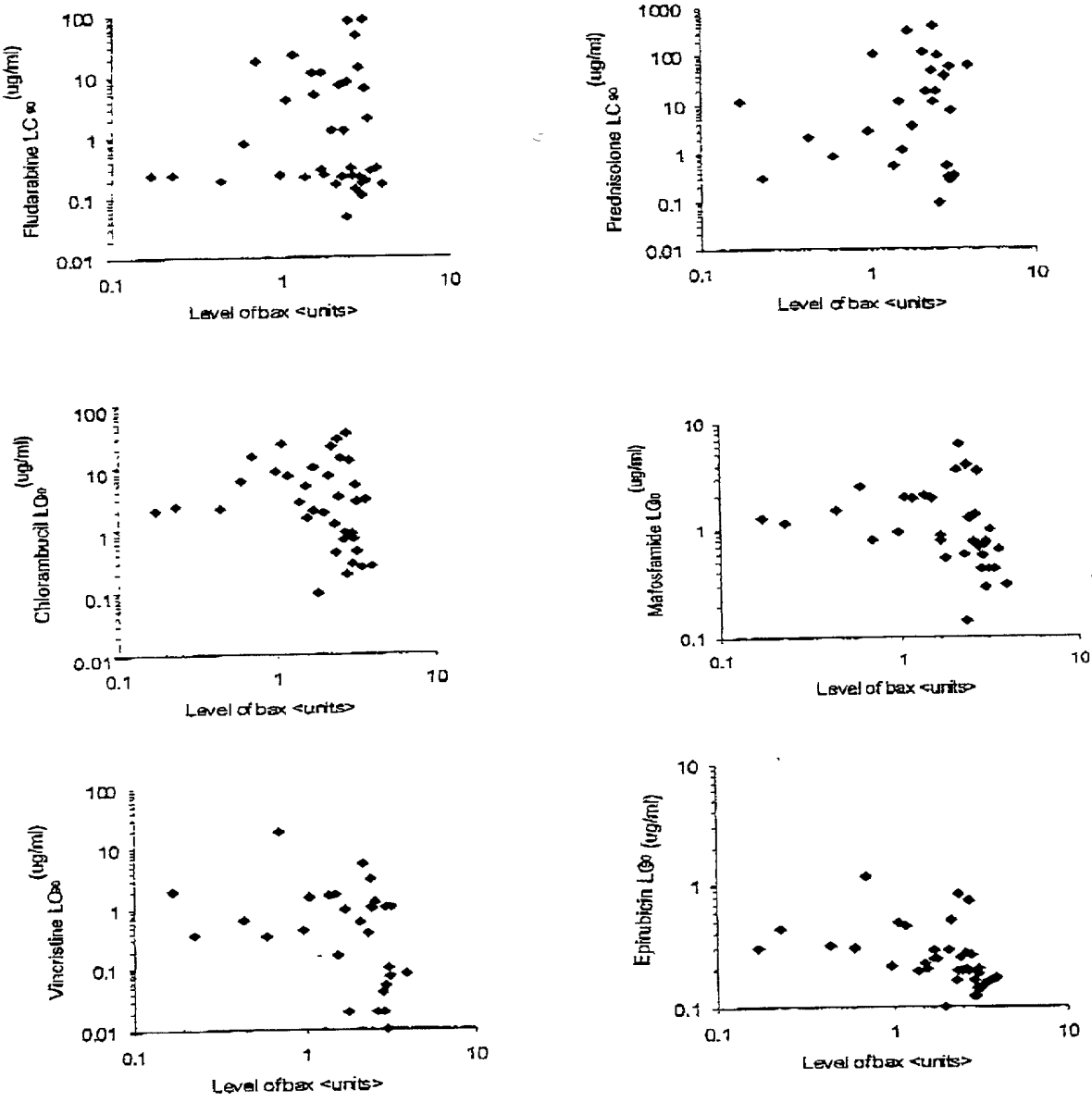
12. Use in accordance with claim 11, wherein the status, namely the expression profile and/or mutations of genes of the Bcl-2 family, mainly Bax, p53, p16, caspases, Rb, cyclins, inhibitors of the cyclin-dependent kinases (CDKIs), ATM and inhibitors of the apoptosis proteins (IAPs), of individual genes or in different combinations is determined.

13. Use in accordance with claim 12, wherein the status of Bax expression or mutations of the Bax gene or Bax promoters and Bax regulators is used.

14. Use in accordance with claim 12, wherein the status of the p53 expression or mutations of the p53 gene is used.

15. Use in accordance with claim 11 to 14, wherein the status of Bax and p53 risk-adapted tumor therapies with malignant hematological diseases, like CLL, and other tumoral diseases is used.

16. Use in accordance with claim 11 to 15 of a combination of p53 and Bax and possibly other cell cycle and apoptosis regulators either alone or in their combination in the sense of a molecular pathway diagnosis with malignant tumors or precancers.


Fludarabine LC90 (ug/ml)
100
10
1
0.1
0.01
0.1
1
10
Level of bax <units>
Prednisolone LC90 (ug/ml)
1000
100
10
1
0.1
0.01
0.1
1
10
Level of bax <units>
Chlorambucil LC90 (ug/ml)
100
10
1
0.1
0.01
0.1
1
10
Level of bax <units>
Mafosfamide LC90 (ug/ml)
10
1
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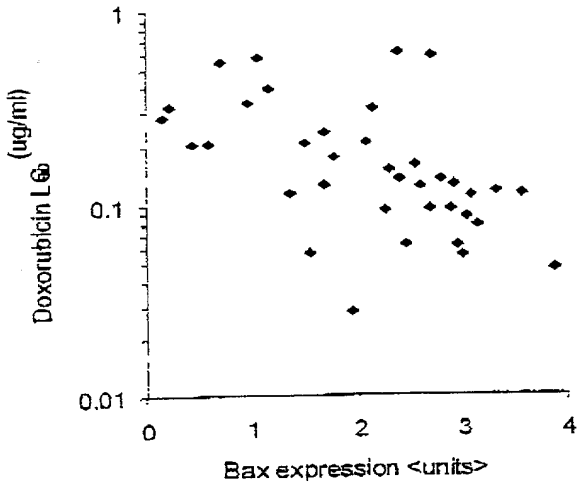
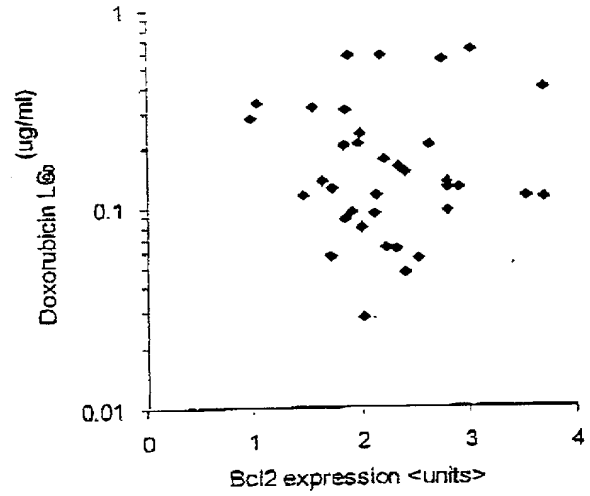
0.1
1
10
Level of bax <units>
Vincristine LC90 (ug/ml)
100
10
1
0.1
0.01
0.1
1
10
Level of bax <units>
Epirubicin LC90 (ug/ml)
10
1
0.1
0.1
1
10
Level of bax <units>

FIG. 1


1
0.1
0.01
Doxorubicin LC50 (ug/ml)
0
1
2
3
4
Bax expression <units>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0.01
Doxorubicin LC50 (ug/ml)
0
1
2
3
4
Bcl2 expression <units>

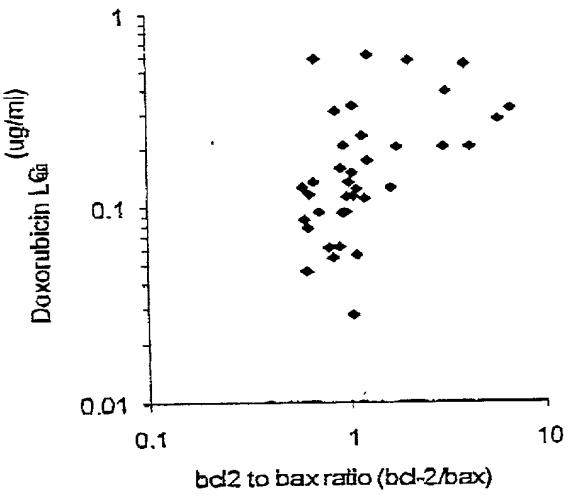
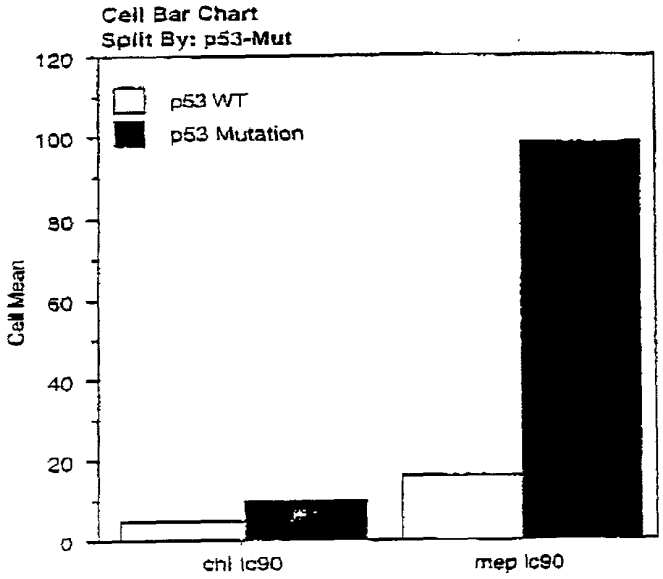

1
0.1
0.01
Doxorubicin LC50 (ug/ml)
0.1
1
10
bcl2 to bax ratio (bcl-2/bax)

FIG. 2

A Effect of the p53-mutation on the sensitivity to Chlorambucil and Melphalan



B Effect of the p53-mutation on the sensitivity to Fludarabine

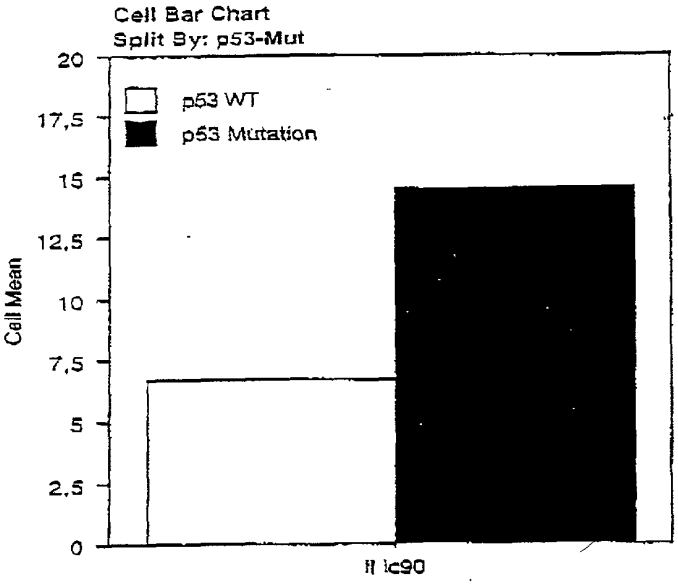


FIG. 3

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If each inventor understands English, the Declaration and Power of Attorney below is suitable for use when filing a regular patent application and also when entering the national stage, in the case of an International application designating the USA under the PCT.

COMBINED DECLARATION AND POWER OF ATTORNEY FOR PATENT APPLICATION	Attorney Docket No. 101195-67

As a below named inventor, I hereby declare that:
My residence, post office address and citizenship are as stated below next to my name,
I believe I am the original, first and sole inventor (if only one name is listed below at 201) or an original, first and joint inventor (if plural names are listed below at 201-205) of the subject matter which is claimed and for which a patent is sought on the invention entitled

Method for Detecting the Effect of Different Chemotherapeutic Agents and/or Radiation Therapy in Malignant Diseases and Method for Selecting Therapeutic Agents for the Therapy Thereof

the specification of which (check one)

____ is attached hereto

__✓__ was filed on ____10 May 2000______

under Serial Number ___PCT/DE00/01444__ and was amended on ____________________ (if applicable).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, Section 1.56.

I list below any prior foreign application(s) for patent or inventor's certificate in respect of which foreign priority benefits are claimed under 35 USC 119; and any prior foreign application(s) for patent or inventor's certificate in respect of which such foreign priority rights are not claimed and which has a filing date before that of any application in respect of which such foreign priority benefits are claimed:

Application Number	Country	Filing Date (day, month, year)	Priority Claimed under 35 USC 119
199 22 052.2	**Germany**	**14 May 1999**	YES: ✓ NO:____
			YES:____ NO:____
			YES:____ NO:____

I hereby claim the benefit under Title 35, United States Code, §119(e) of any United States provisional application(s) listed below.

Application No.	Filing Date

I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith:

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Signature of Inventor 201 > Peter Jann	Date Jan. 30, 2002
Signature of Inventor 202 > Timo Lillberg	Date Jan. 30, 2002
Signature of Inventor 203 > Bernd Dittler	Date Jan. 30, 2002
Signature of Inventor 204 >	Date Dec. 10, 2001
Signature of Inventor 205	Date